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by

George A. Boyd

University of Rochester

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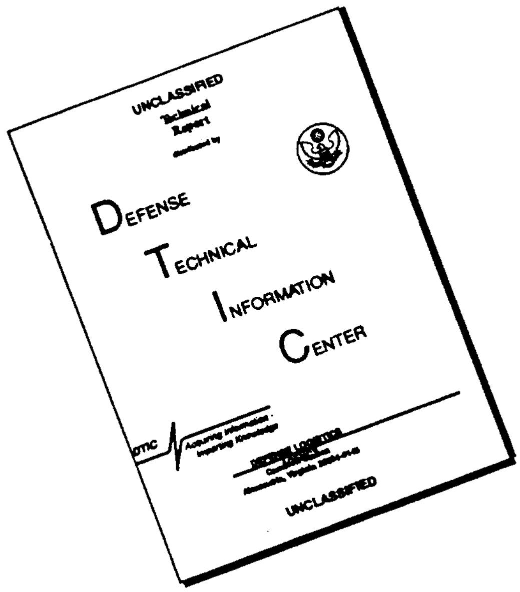

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ABSTRACT

The grain speed of Eastman NTB and medium lantern slide emulsions were compared by exposing to a known number of I^{131} beta particles. Grains were counted. On the basis of this work and unpublished work by the Eastman Kodak Research Laboratories, an I^{131} beta particle has equal probability of rendering a grain developable in the present NTB emulsions as in the medium lantern slide, even though the latter are larger. The NTB fog is low and resolution high.

INTRODUCTION

Bélanger and Leblond[1] introduced medium lantern slide emulsions for histological autoradiographs of beta emitters. As Evans[2] has pointed out, the medium lantern slide has the disadvantage of high stain uptake which reduces the differential of color intensity between the tissue and emulsion background. It is also known that this emulsion has a fog background which, although less than X-ray film used by Hamilton et al,[3] is nevertheless a disadvantage in making precise autoradiographic studies.

In the last twelve months the Eastman Kodak Company has placed on the market a series of plates designed especially for the recording of nuclear particles. One of these, the NTB emulsion originally developed for registering protons and other weakly ionizing particles, viz. mesons and electrons, was found to be the most sensitive to beta particles of all the emulsions of its range of grain size.

We have recently investigated the emulsion stain uptake, speed and background fog of NTB emulsions as compared with the medium lantern slide. Although the NTB plates now available are faster than those from the one emulsion batch studied and hence the speed determinations are already out of date, this preliminary communication seems to be in order to acquaint those interested in autoradiography with the availability and advantages of this new emulsion.

The emulsion is loaded with approximately 80% silver bromide by weight. The mean grain diameter before development is 0.2 – 0.3 micra which develops in D-19 at two minutes and 20°C to a mean of about 0.5 micra. The light sensitivity is very low and it can be handled under a Series II Waratten Safelight.

MEASUREMENT OF GRAIN SPEED AND BACKGROUND

The speed of the NTB emulsion (an early batch, No. 264196, was studied) and of a commercial medium lantern slide to beta particles from I^{131} was measured. In photographic work it is the custom to think of speed in terms of optical densities. In this case speed refers to the probability of a

grain being made developable. A water imbibing Cellophand film, 10μ thick and approximately 25 sp cm in area, was soaked from 10 to 20 minutes in a radio-iodine solution containing approximately 7 microcuries per cc. The Cellophane was removed and rinsed to remove unevenly distributed thicknesses of solution film and placed on a glass plate to slowly dry by evaporation in the absence of air currents. While wet, the Cellophane was pressed to the glass plate at all points of the surface to give uniformity of surface forces and even distribution of the solution to aid in uniform evaporation from the surface. After drying, a small disk of 0.245 inch in diameter was punched out from a large area which appeared to have remained in contact with the glass. With these precautions it was felt that the iodine was uniformly distributed and that any one microscopic field would be reasonably the same concentration as another for the same disk. This was supported by counting the grains in twenty fields of $1,687\mu^2$ each, by which was found a coefficient of variation of 8%. The counting method and precautions are given below.

The rate of disintegration was measured by a Geiger counter for which the counter efficiency for I^{131} betas had been determined. The absolute rate of disintegration was determined from the count, the absorption factor, and the known geometry of the instrument. The disk was then placed on the photographic plate and held in close contact with a brass slab.

After development, the grains were counted with bright field illumination with a 1.8 mm objective and a 10X ocular. All counting was done in a room where the only illumination was the microscope lamp.

To determine the counting error, eye fatigue and reproducibility without fatigue were determined. The former was tested by dividing a field into three equal areas and successively counting the three areas before resting the eyes. The coefficient of variation did not change from areas number one to three for bright field. A total of approximately 225 grains was counted.

With this known number of grains countable without eye fatigue, the reproducibility was determined. A field of $1,687\mu^2$ was counted twenty times, resting the eyes after counting about 200 grains. The coefficient of variation for the technician making all the counting was found to be 2.6%.

In the final count the number of grains counted before resting the eyes was reduced to 150 to be on the safe side of the error.

The same Cellophane disk was used for the NTB and the medium lantern slide emulsions. By this method it was found the medium lantern slide required on the average the equivalent of 10 beta particles to render a grain developable under the conditions stated above. This does not imply that 10 beta particles must hit a single grain. For example, some betas would probably pass through the emulsion without rendering any of the grains developable, while on the other hand a beta particle at the tail end of its track when most of its energy is spent will be moving slowly enough to produce several developable grains in its passage through the emulsion.

The NTB emulsion required the equivalent of 18 or approximately twice the number of beta particles per grain. These values have not been corrected for scattering from the brass above the Cellophane disk or the glass below the emulsion.

Thus the medium lantern slide grain speed is approximately twice that of this particular NTB emulsion batch. As mentioned earlier, however, the presently available NTB emulsions are faster. According to the Eastman Kodak Research Laboratories the present NTB emulsions as tested by X-rays are approximately twice as fast as this NTB emulsion batch. Therefore, the presently available NTB emulsions should be approximately equivalent in speed to the medium lantern slide.

The above values give a measure of the probability of a grain being rendered developable by a beta particle. In practice, however, one is not concerned with a single layer of grains since an emulsion has several layers stacked one upon the other. The mean distribution of silver bromide grains in an NTB emulsion is at least* one per cubic micron, and thus an emulsion of 10 micra thickness

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* The exact concentration has not yet been measured by the Eastman Kodak Research Laboratories. It is known, however, that the minimum is as stated.

would contain on the average a depth of ten grains. Thus, a 10 micra emulsion should provide the ten grains in the NTB emulsion to record about one-half of the betas from I^{131}. If the newer NTB emulsions prove to respond to I^{131} betas similarly to electrons from X-rays, we should then expect in 10 micra a number of silver grains equivalent to the number of betas passing through it if the number is sufficiently large to be statistically significant.

An outstanding advantage of the NTB emulsion over the medium lantern slide is the low fog or background grains. The fog measurements were made on the same plates from which the speeds were determined. The fields for counting fog grains were more than 1 cm from the edge of the image produced by the Cellophane disk containing the radio-iodine. With a similar counting technique as described above, the NTB emulsion was found to have approximately 0.05 fog grains per $100\mu^2$ of an emulsion 9μ thick, whereas the medium lantern slide had 8.5 grains per $100\mu^2$ for emulsion 15μ thick. The medium lantern slide has approximately 100 times the background fog of this particular NTB emulsion for equal emulsion thickness.

STAIN UPTAKE

Because of the high loading factor, the concentration of gelatin is reduced over the medium lantern slide emulsions and the stain uptake is much less. Thus, for emulsions of equal thickness the NTB tissue autograph will give a greater stain differential between the tissue and the emulsion.

We have in this laboratory also reduced the stain uptake by using thin emulsions which were kindly prepared by the Research Laboratory of the Eastman Kodak Company. The thin emulsions are available in thicknesses down to 5μ. They give the additional advantage of increased resolution; however, all is not gain since effective plate speed is decreased as the thickness is decreased. A 10μ emulsion seems to be a fair compromise for speed, stain uptake, and ease of manufacture. If one wishes to eliminate staining of the emulsion entirely, stripping film can be used wherein the tissue is placed on the emulsion base instead of on the emulsion.[4] The emulsion is then protected by parafining the edges during staining of the tissue.

DISCUSSION

Figure 1 shows the concentration of silver grains in the images in two emulsions and in the backgrounds of three. Since some experimenters have used type K X-ray emulsion because of its high speed, the background fog produced only by development is included. The NTB and type K emulsions were developed in D-19 and the medium lantern slide in D-72 as recommended.

All photographs of Figure 1 were taken originally with a 1.8 mm objective and a 10X ocular. Since the depth of the field of this objective is about 0.5μ and the NTB grains are only about 0.5μ in diameter, a very small number are in sharp focus. Most of them appear as diffraction halos. The grains in the medium lantern slide are larger and hence a part of a larger number falls within the shallow depth of the field and appear in focus. The same is true for the K X-ray emulsion.

Another interesting point illustrated by 1-A and 1-B are the very large grains. These are due to clumping caused by the heavy loading of silver bromide. This objection has been overcome to a large extent by the newer NTB emulsions.

It is evident that with the low number of fog grains in the NTB emulsion, the presence of grains produced by beta particles can be determined with greater certainty than for the medium lantern slide. In effect then, the low fog enhances the speed of the NTB even more when compared with the medium lantern slide. Because of this extremely low fog, the NTB emulsion offers promise of a fair quantitative method of measuring beta emitters in a tissue by counting the individual grains.

Since every emulsion batch differs, each batch of plates should be calibrated both for sensitivity and for fog. Furthermore, the plates should be kept under ideal conditions, i.e., in an airtight, lead

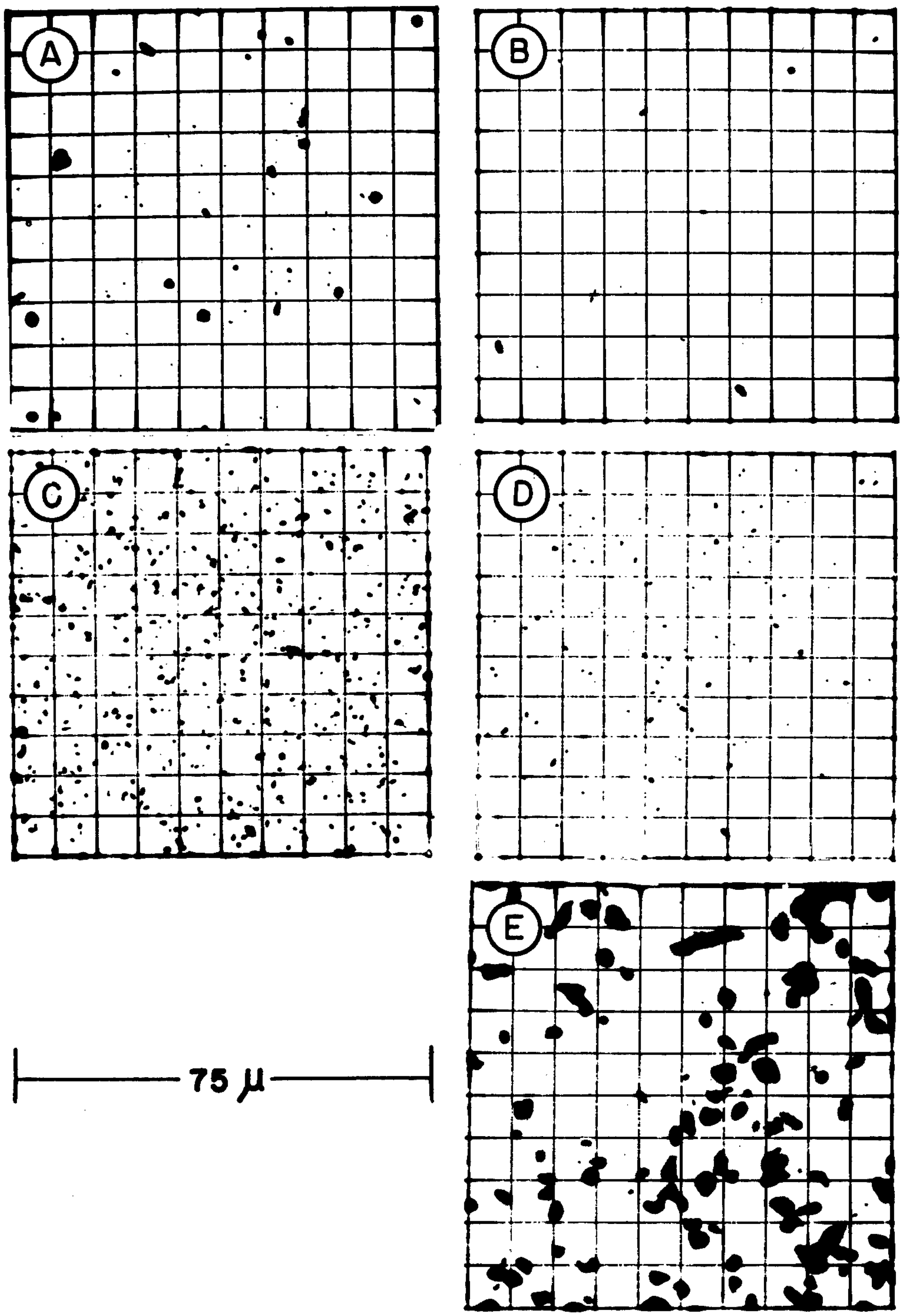

Figure 1. A. Concentration of silver grains in a just visible image produced by I^{131} betas in 9μ thick NTB emulsion. B. Background fog in the same NTB emulsion. C. Concentration of silver grains in an image (visibility greater than in A) produced by I^{131} betas in a 15μ thick medium lantern slide emulsion. D. Background fog in the same medium lantern slide emulsion. E. Background grains in K X-ray emulsion.

lined, dark box at ice box temperature to prevent the increase in fog due to stray radiation or chemicals before use or during autoradiographic exposure.

Figure 2 shows autoradiographs of a section of rat thyroid containing I^{131}. One-half a millicurie of I^{131} was injected into a rat which was sacrificed 24 hours later. After about a week's delay for fixing and embedding, a tissue section was laid on an NTB emulsion of 9μ thickness according to the method of Endicott[5] and of Evans.[6] This figure shows an exposure for 17 hours. To show both the tissue and the autoradiograph at a magnification of 970X, it is necessary to photograph the tissue just above the tissue-emulsion interface and the autograph of individual grains just below the interface. Figure 2-A shows the tissue and 2-B the autograph.

The high resolution of this emulsion is shown by the autoradiographic bridge encircled in Figure 2-A corresponding to a colloid bridge also encircled in 2-B. Figure 3 shows another autoradiograph of this same thyroid for an exposure of 165 hours.

In conclusion, on the basis of our measurements of the early NTB emulsion and the comparison by the Eastman Kodak Research Laboratories of the latest NTB emulsions and medium lantern slides, the grains of the NTB emulsions now available are approximately equivalent in sensitivity to high energy beta particles as those of the medium lantern slide. And if the fog proves to be equivalent to the fog of this early batch which has been measured, it would have two advantages over the medium lantern slide: one, giving a higher stain differential between the tissue and emulsion, and, two, giving a lower background fog to enhance the certainty of location and measure of radioactive beta emitters in tissue.

ACKNOWLEDGMENT

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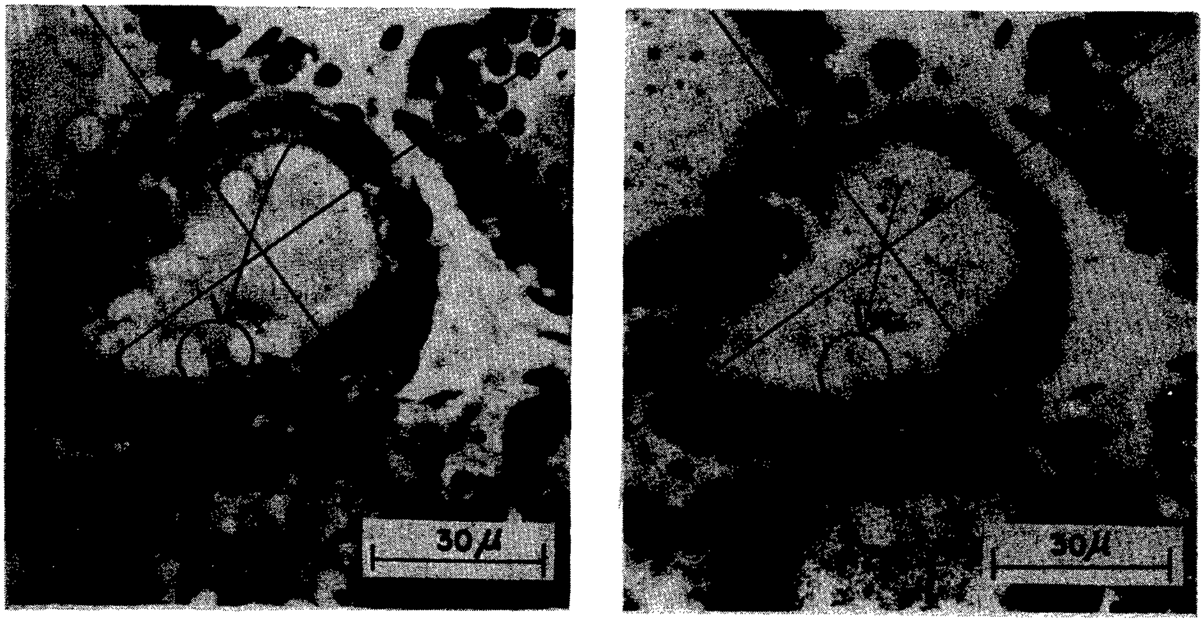
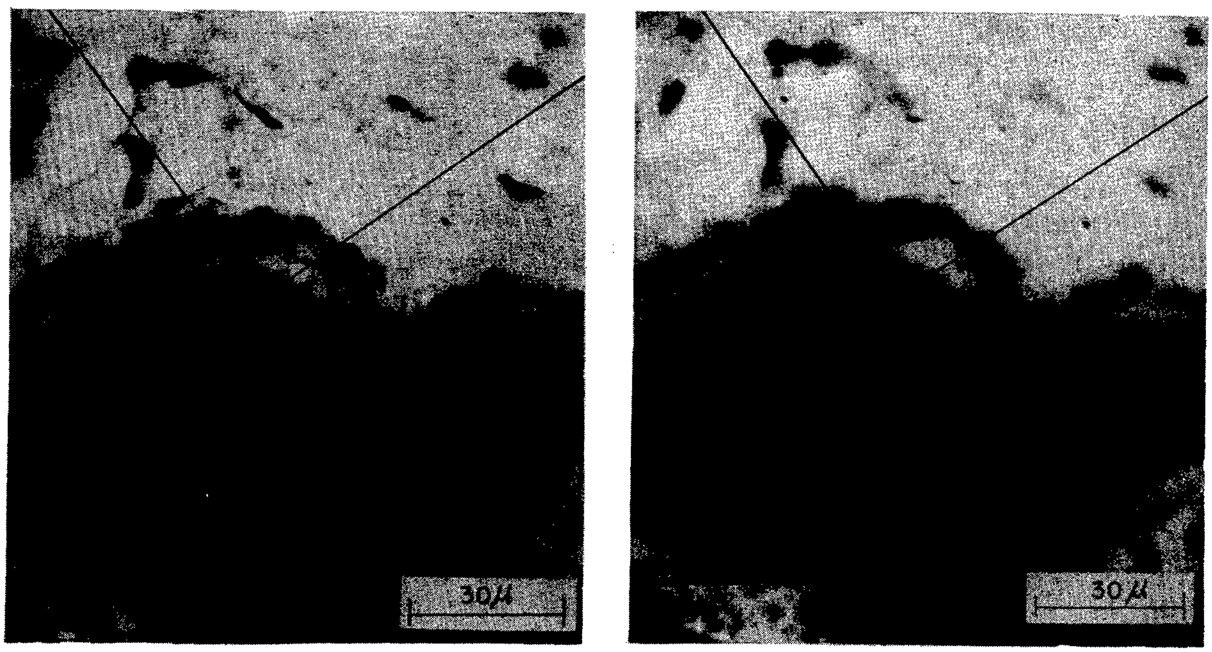


Ⓐ TISSUE LEVEL

Ⓑ GRAIN LEVEL

Figure 2. Photomicrographs of a thyroid tissue-autograph. 17 hour exposure on NTB emulsion plate of 7μ section containing I^{131} from rat sacrificed 24 hours after injection of 500 μc.



Ⓐ TISSUE LEVEL

Ⓑ GRAIN LEVEL

Figure 3. Same as Figure 2 with 165-hour exposure.

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